

migration in eukaryotes. The building block for MT polymerization is the  $\alpha$ -tubulin heterodimer, which is present as a tightly regulated soluble pool in the cytoplasm. Despite the importance of  $\alpha$ -tubulin heterodimer in the regulation of MT dynamics, it remains unclear how nascent and folded  $\alpha$  and  $\beta$ -tubulin are assembled and activated into a single heterodimer configuration that universally dictates dynamic MT polymerization. It remains unknown how five conserved tubulin cofactors (TBC-A,B,C,D and E) and a dedicated Arl2 G-protein promote  $\alpha$ -tubulin biogenesis, activation and degradation, and how such activities impact MT function. In contrast to a long-standing hypothesis in which individual tubulin cofactors bind sequentially to  $\alpha$  and  $\beta$ -tubulin monomers and assemble  $\alpha$ -tubulin dimers through dynamic interactions, we show based on biochemical and structural studies that multiple tubulin cofactors and Arl2 form multi-subunit platforms for  $\alpha$ -tubulin dimer assembly, activation and degradation. We show that multi-subunit tubulin cofactor and Arl2 platform are soluble  $\alpha$ -tubulin regulators that are powered by GTP hydrolysis cycles. We have determined tubulin cofactor platform structure, conformational changes upon tubulin dimer binding, and the mechanism of GTP hydrolysis activation. Surprisingly, we further show, using reconstitution of these complexes with soluble tubulin dimer and dynamic MTs, that they enhance  $\alpha$ -tubulin polymerizing state at MT plus ends in a manner dependent on Arl2 and tubulin GTP hydrolysis. Our data surprisingly suggest tubulin cofactors are potent regulators of soluble tubulin dimer state, and promote soluble tubulin activation required for all MT dynamics. Our model explains long-standing cell biology and genetics data about the roles of tubulin cofactors in regulating the soluble  $\alpha$ -tubulin pool and MT homeostasis.

#### 2238-Plat

##### Structural Basis for Nucleotide Exchange and Power Stroke Generation by the Kinesin Molecular Motor

Zhiguo Shang<sup>1</sup>, Roseanne Csencsits<sup>2</sup>, Chen Xu<sup>3</sup>, Jared C. Cochran<sup>4</sup>,

Charles Vaughn Sindelar<sup>1</sup>.

<sup>1</sup>Yale University, New Haven, CT, USA, <sup>2</sup>Lawrence Berkeley National Laboratory, Berkeley, CA, USA, <sup>3</sup>Brandeis University, Waltham, MA, USA,

<sup>4</sup>Indiana University, Bloomington, IN, USA.

Kinesin molecular motors use energy derived from ATP to step along microtubules, driving many essential processes in eukaryotic cells, including mitosis, vesicle transport and cytoskeletal remodeling. Several conformational states of kinesin have been identified by X-ray crystallography, but the structural transitions used by kinesin to generate force and movement along intact microtubules have remained unclear. We used recent improvements in cryo-EM methodology and instrumentation to capture the conformation of microtubule-attached kinesin at the beginning (no-nucleotide) and end (ATP analog-bound) of the force generation process at 5-6 Å resolution. We derived all-atom models for these two maps from a crystal structure of the tubulin-kinesin complex, using explicitly solvated molecular dynamics simulations combined with restraints derived from the maps. This analysis revealed that, contrary to existing models, kinesin's central beta sheet serves as the primary transducer in the motor's force-generation mechanism, twisting to drive ADP release and subsequently untwisting upon ATP binding to trigger a power stroke. We identified conserved residues on the motor domain, supported by additional structural and biochemical analysis of site-directed mutations, which serve as allosteric latches during the motor's microtubule-attached phase. These latches regulate the sheet-twisting motion and couple key properties of motor function to each other, including nucleotide binding, hydrolysis, and generation of a power stroke. These findings reveal how interactions with the microtubule can fundamentally alter kinesin's energetic landscape in order to initiate productive motility.

#### 2239-Plat

##### Bimodality in a System of Active and Passive Kinesin-1 Motors

Lara Scharrel<sup>1</sup>, Rui Ma<sup>2,3</sup>, Frank Jülicher<sup>4</sup>, Stefan Diez<sup>1,5</sup>.

<sup>1</sup>B CUBE, TU Dresden, Dresden, Germany, <sup>2</sup>Max Planck Institute for the Physics of Complex Systems, Dresden, Germany, <sup>3</sup>Institute for Advanced Study, Tsinghua University, Beijing, China, <sup>4</sup>Max Planck Institute for the Physics of Complex System, Dresden, Germany, <sup>5</sup>Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany.

Long-range directional transport in cells is facilitated by microtubule-based motor proteins. One example is transport in a nerve cell, where small groups of motor proteins, such as kinesin and dynein, work together to perform the supply and clearance of cellular material along the axon. Defects in axonal transport have been linked to Alzheimer and other neurodegenerative diseases. In particular two diseases, Hereditary Spastic Paraplegia (HSP) and Charcot-Marie-Tooth type 2A neuropathy (CMT2A) are connected to mutations of kinesin family members in the motor domain that affect their ATPase activity. However, it is

not known how in detail multi-motor based cargo transport is impacted if the motor function of a fraction of motors is inhibited. In order to mimic hindered multi-motor transport in-vitro, we performed gliding motility assays with varying fractions of active kinesin-1 and passivated kinesin-1 (rigor mutants). We found that hindered gliding manifests in three motility regimes: gliding at the velocity of single motors, simultaneous gliding and stopping (bistable movement), and stopping. Notably, an abrupt transition from gliding to stopping occurred at a certain threshold fraction. Furthermore, we developed a theoretical description based on single motor parameters. Our model explains the bimodal microtubule movement as well as the sharp transition from gliding to stopping. Our results demonstrate that hindered transport is acting in a bimodal "either-or-fashion": depending on the fraction of passive motors, transport by a multi-motor system is either performed close to full speed or not at all.

#### 2240-Plat

##### A Comparative Study of the Major Biochemical States of Kinesin-MT Complex using Computational Techniques and All-Atom Structural Models

Srirupa Chakraborty, Wenjun Zheng.

Biophysics, University at Buffalo, Buffalo, NY, USA.

Here we apply computational molecular dynamics simulation techniques to do a comprehensive comparative study of the three major biochemical states of a kinesin - microtubule (MT) complex. These states are namely, the nucleotide-free APO state, the ADP-bound state and the ATP-bound state. We built atomistic structure models for these key MT-binding states with different nucleotide content using available crystal structures, homology modeling and flexible fitting of high-quality cryo-electron-microscopy (EM) maps. We next explore how MT binding modulates active site dynamics of kinesin, predict some of the structural changes and pin-point some of the key residues that control the transition states. We further study the binding free-energy between kinesin head and MT in the three states and also identify a list of interactions (hydrogen bonds and salt bridges) between kinesin and MT and also between kinesin and ligand (ADP). We further perform steered molecular dynamics to mimic the intramolecular strain and identify some residues in force regulation of binding affinity. This study helps us identify promising targets for future mutational and functional studies of the kinesin-MT complex.

#### 2241-Plat

##### Self-Regulation of Cytoplasmic Dynein through its Unconventional Force Response

Takayuki Torisawa<sup>1</sup>, Ken'ya Furuta<sup>2</sup>, Akane Furuta<sup>2</sup>,

Muneyoshi Ichikawa<sup>1</sup>, Kei Saito<sup>1</sup>, Kazuhiro Oiwa<sup>2,3</sup>, Hiroaki Kojima<sup>2</sup>,

Yoko Yano Toyoshima<sup>1</sup>.

<sup>1</sup>Department of Life Sciences, Graduate School of Arts and Sciences, The University of Tokyo, Tokyo, Japan, <sup>2</sup>Advanced ICT Research Institute, National Institute of Information and Communications Technology, Hyogo, Japan, <sup>3</sup>Graduate School of Life Science, University of Hyogo, Hyogo, Japan. Cytoplasmic dynein is essential for a wide range of cellular activities, including intracellular transport, cell division, and cell migration in most of eukaryotic organisms. To achieve these various functions, dynein activity must be tightly controlled. However, the motility of mammalian cytoplasmic dynein has been controversial in previous studies, which makes it unclear how dynein activity is regulated and tuned for a specific function. Here, using electron microscopy and DNA nanostructure-based motility assays, we investigated how the control of dynein activity is achieved. We showed that single dynein molecules diffused along microtubules in an autoinhibited state, in which two motor heads were stacked together. This state was released when multiple dynein molecules worked together on a single cargo or when dynein was pulled by an optical tweezer, suggesting that individual dynein molecules in the team were activated through destabilization of the stacked conformation by mechanical strain generated between dynein molecules. We confirmed this force-dependent activation mechanism by observing the movement of a chimeric dynein fused with an inactive kinesin, which we assume acts as a load. This mechanism would function at a fundamental level of dynein regulation that does not require an external regulator, thereby serving as a stable basis for the higher-level regulation of dynein-driven transport.

#### 2242-Plat

##### Dynein's C-Terminal Domain Plays a Novel Role in Regulating Force Generation

Peter Höök<sup>1</sup>, Matthew P. Nicholas<sup>2</sup>, Sibylle Brenner<sup>2</sup>, Caitlin Lazar<sup>1</sup>,

Sarah J. Weil<sup>1</sup>, Richard B. Vallerie<sup>1</sup>, Arne Gennerich<sup>2</sup>.

<sup>1</sup>Columbia University College of Physicians and Surgeons, New York, NY, USA, <sup>2</sup>Albert Einstein College of Medicine, Bronx, NY, USA.

Cytoplasmic dynein is a homodimeric, minus-end-directed microtubule motor protein involved in a wide range of both low and high force requiring functions